Karyotyping of transformed human epithelial cells from exposures of heavy ions

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About Me

- PhD candidate at Texas southern university.
- Currently My research focus is on Genomic Instability.
- My interest is to pursue a carrier in science and research

Why I am interested in NASA:

- I am interested in NASA because of my exposure to research at Johnson Space Center as graduate co-op .
- Opportunity to work with a team of highly experienced scientists
- To contribute my part in the understanding the risk of long term space flight mission.

Objectives of the research

- Understanding chromosomal instability in the transformed clones from space radiation exposure.

Background

- Why NASA is interested ?

- Heavy ions create a potential health risk to astronauts who have participated in extended space missions. Some of the biological effects, including cell inactivation, genetic mutations, cataracts and cancer. (Honglu Wu 2007)

- Most of these biological end points are closely correlated with chromosomal damage, which can be used as a biomarker for radiation damage. (Honglu Wu 2007)

Radiation

- There are three forms of Radiation in space

Trapped radiation,
solar particle events (SPE),
galactic cosmic rays (GCR),

- GCR is mostly comprised of atoms ranging from protons to that of high atomic number (Z) atoms such as iron (56Fe-) that have had their electrons stripped off while being accelerated to high rates of speed.

(Curtis and Letaw, 1989; National Council on Radiation Protection and Measurements., 2006).

Genomic instability

- Its refers to an increased tendency of alterations in the genome during the life cycle of cells. It's a major driving force for almost all human cancer.
- Often, after being damaged by radiation, cells are able to repair DNA damage and reproduce normally.
- However, sometimes damage may carry over for several generations before the unobserved damage causes the cell to lose control of its genome.
- At this point, cells may be unable to reproduce successfully. They may become genetically unstable, or become cancerous.
- Previous study conform the Tumor cells have double the chromosome number. In tumorigenesis, aneuploidy is frequently preceded by tetraploidy. Major issues include how tetraploidy arises and how cells can effectively respond to this state.

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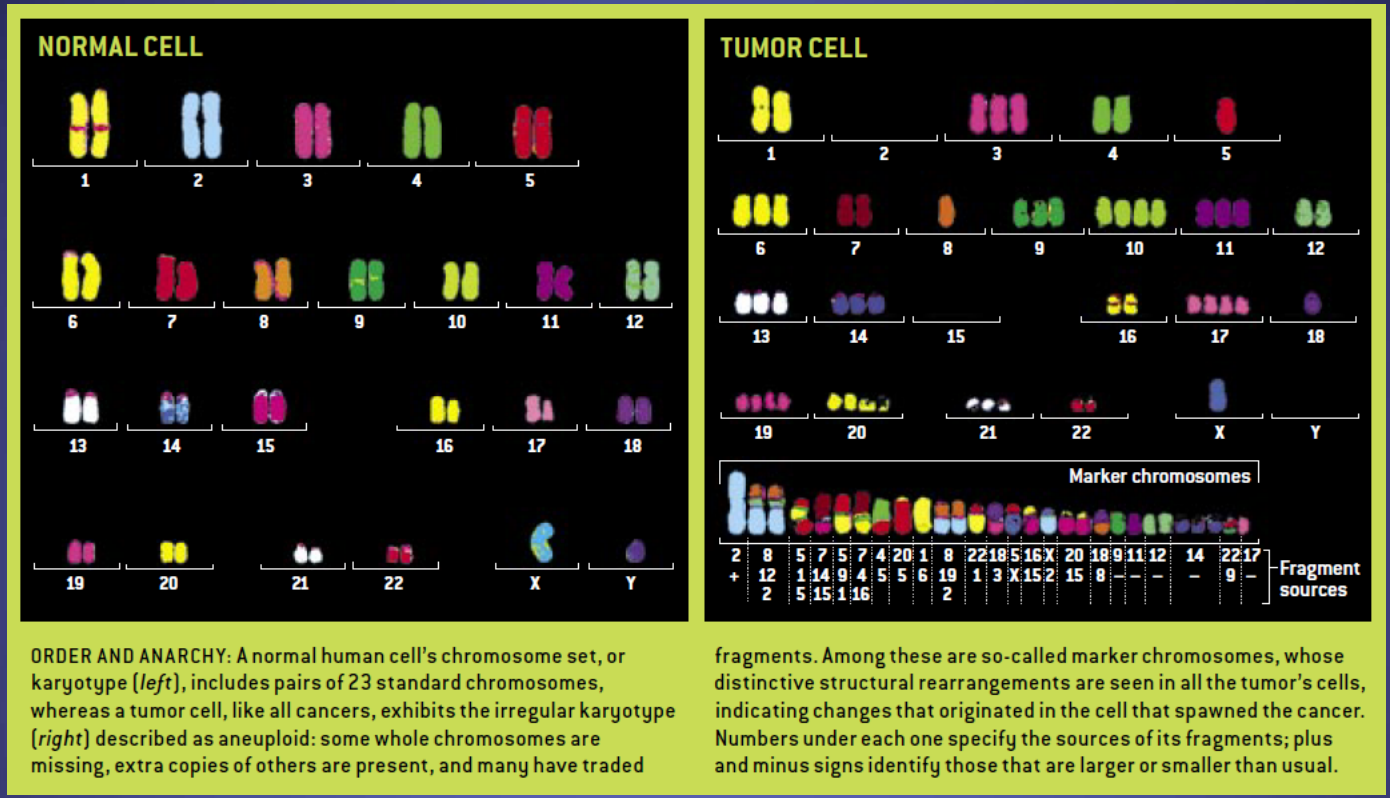
Chromosome aberrations in cancer cells



ORDER AND ANARCHY: A normal human cell's chromosome set, or karyotype (*left*), includes pairs of 23 standard chromosomes, whereas a tumor cell, like all cancers, exhibits the irregular karyotype (*right*) described as aneuploid: some whole chromosomes are missing, extra copies of others are present, and many have traded fragments. Among these are so-called marker chromosomes, whose distinctive structural rearrangements are seen in all the tumor's cells, indicating changes that originated in the cell that spawned the cancer. Numbers under each one specify the sources of its fragments; plus and minus signs identify those that are larger or smaller than usual.

Duesberg, Scientific American, 2007

Genomic instability: An increased tendency of the GENOME to acquire MUTATIONS when various processes involved in maintaining and replicating the genome are dysfunctional.

16226–16231 | PNAS | December 10, 2002 | vol. 99 | no. 25

The role of chromosomal instability in tumor initiation

Martin A. Nowak[†‡], Natalia L. Komarova[†§], Anirvan Sengupta[¶], Prasad V. Jallepalli[‖], Ie-Ming Shih[**], Bert Vogelstein[**††], and Christoph Lengauer[‖]

- Chromosome instability (CIN) is a defining characteristic of most human cancers.
- Mutation of CIN genes increase the probability that whole chromosome or large fractions of chromosomes are gained or lost during cell division.
- The consequence of CIN is an imbalance in the number of chromosomes per cell (aneuploidy) and enhanced rate of loss of heterozygosity.
- Any genetics instability, is an early event and consequently a driving force for tumor progression.

Radiation-induced genomic instability

Elevated or persistent genetic damages in cells occurring many generations after exposure

NATURE · VOL 355 · 20 FEBRUARY 1992

Transmission of chromosomal instability after plutonium α-particle irradiation

M. A. Kadhim, D. A. Macdonald, D. T. Goodhead, S. A. Lorimore, S. J. Marsden & E. G. Wright*

Epithelial Cell Line

- Normal human bronchial epithelial cells (HBECs), immortalized through the expression of Cdk4 and hTERT, provide a sustainable cell reagent for the evaluation of the radiation effects *in vitro*.

- HBECs retain a novel multipotent capacity *in vitro (capable of differentiating into both central and* peripheral lung cell types) and thus may represent an unrestricted progenitor of the adult lung that resembles an embryonic progenitor.

Specific Aim of this research

- To investigate chromosome aberrations in epithelial cells (3kt HBEC) due to heavy ions radiation.

Sample overview

- UT Southwestern
- HBEC3-KT cells were irradiated with Fe 0.5 Gy at BNL.

  These cells were brought back to Dallas and maintained in the growth condition for four months.
- After 4 months of culture, the soft agar assay was performed. From that soft agar dish, two individual clones were selected.

UT Southwestern samples

- **Control , HBEC3-KT**
- parental cells

- **Clone # 1**
- Soft agar clone from unexposed HBEC3-KT cells

- **Clone # 2**
- Soft agar clone from unexposed HBEC3-KT cells
- The cells grow very slowly

- **Clone #3**
- Soft agar clone from HBEC3-KT cells with Fe 0.5Gy irradiation.

- **Clone #4**
- Soft agar clone from HBEC3-KT cells with Fe 0.5Gy irradiation.
- The cells grow very slowly

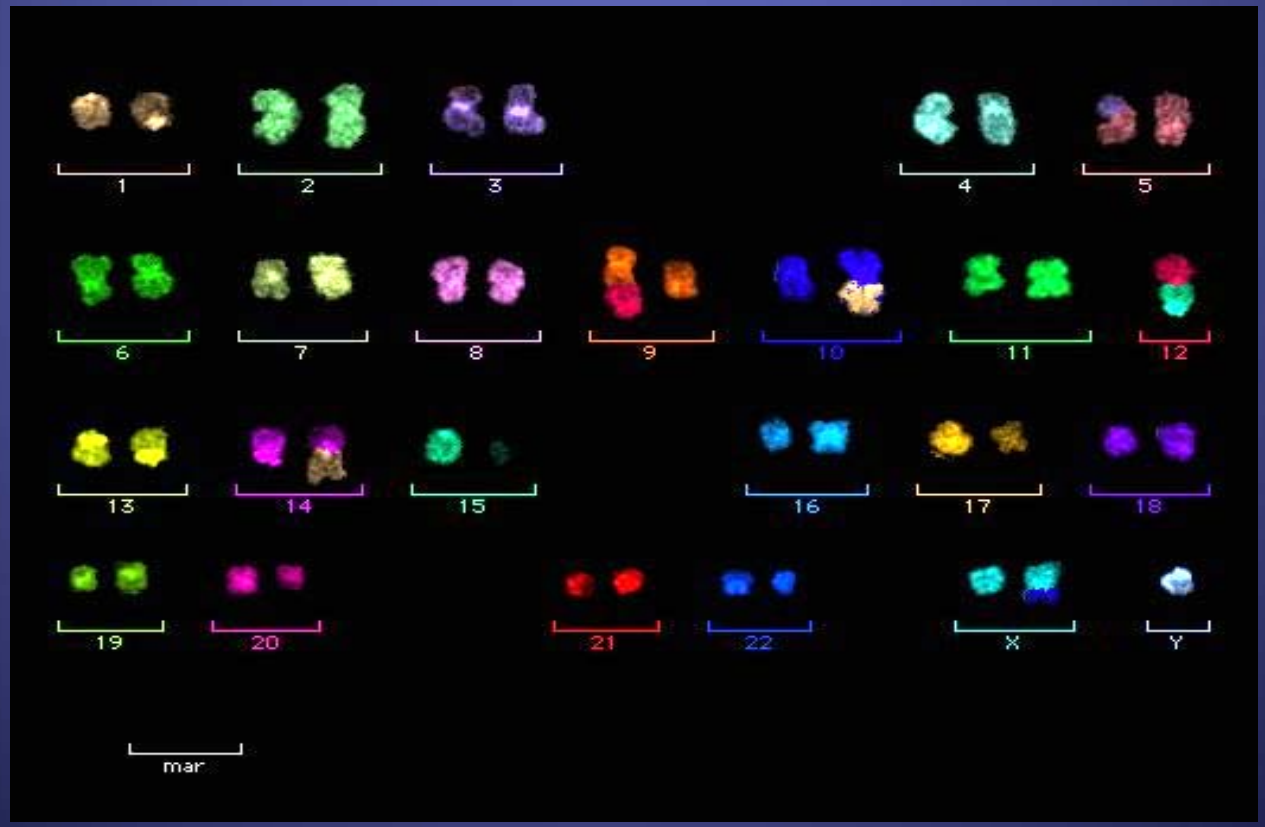
Chromosome preparation

- The cells were split at 80% confluence and cultured in T-75 flasks. They were fed twice a week, subculture once a week, until reaching 80% confluence before the chromosomes were prepared.

- Chromosomes were prepared by dropping fixed metaphase cells onto clean, wet slides. Chromosomes were hybridized *in situ* using the mFISH and mBAND kit (MetaSystems, Altlussheim, Germany) for human chromosome following the protocol recommended by the manufacturer.
- Typical mFISH analysis was performed with all of the chromosomes painted in multiple color bands . *Analyzed all the* chromosomes to see the chromosome aberration.
- mBAND analysis was also performed on one of the clones.

Chromosome painting with Multicolor fluorescence in Situ Hybridization

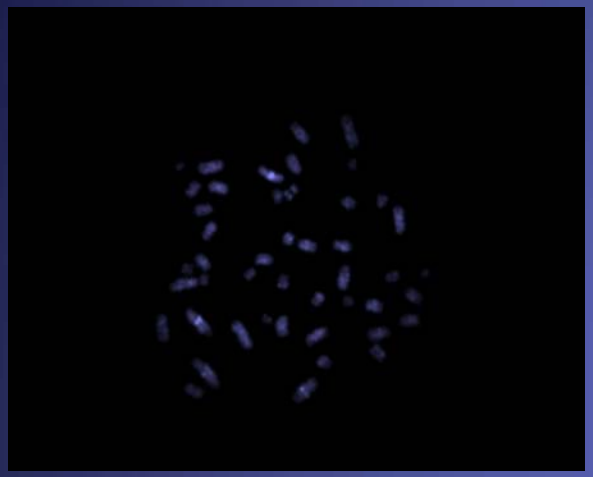
Simultaneous “paints” all 24 different human chromosomes with one single hybridization

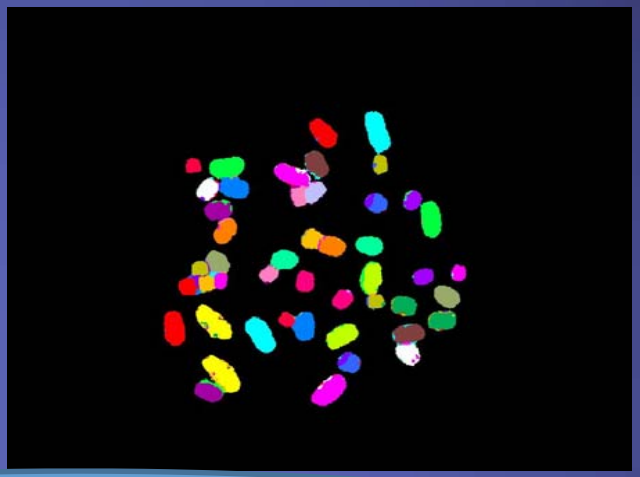


Multicolor Banding *in situ* Hybridization

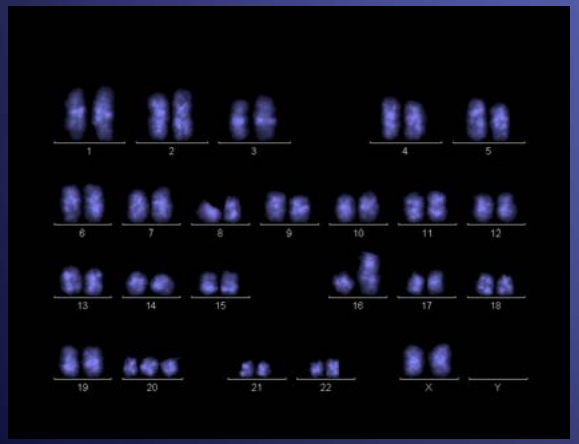
- Color banding pattern along one chromosome
- Higher level of precision within one chromosome
- Detection of intrachromosomal rearrangements
- Detection of break points

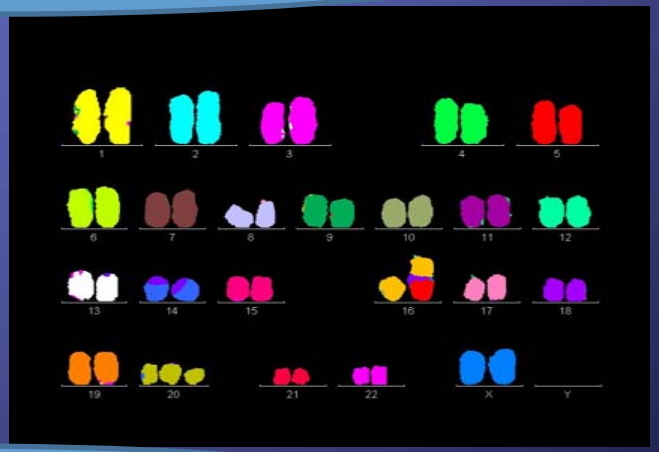
mFISH





Metaphase chromosomes





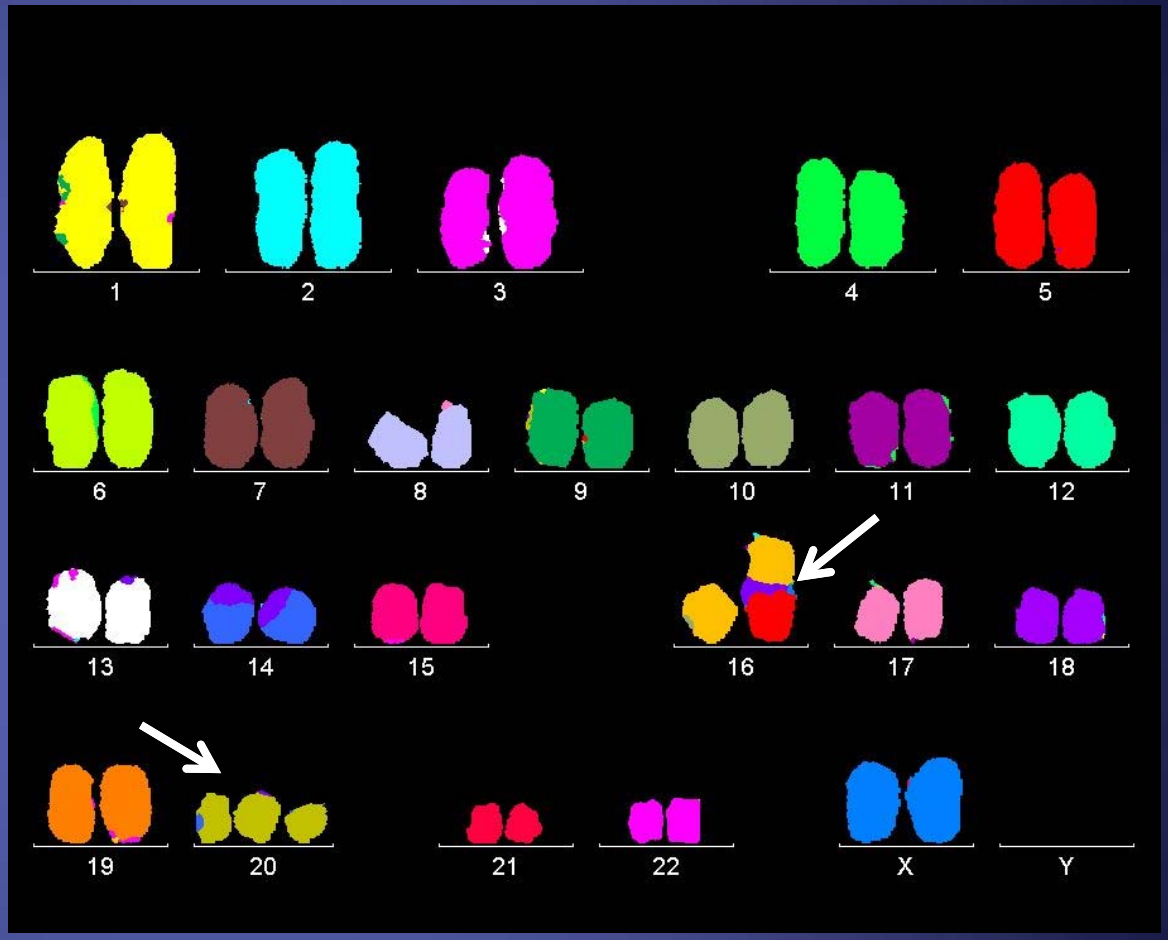
Karyotyped chromosomes

Result

Parental control

- Translocation that results in fusion between chromosomes 5 and 16

- Chromosome 20 has trisomy



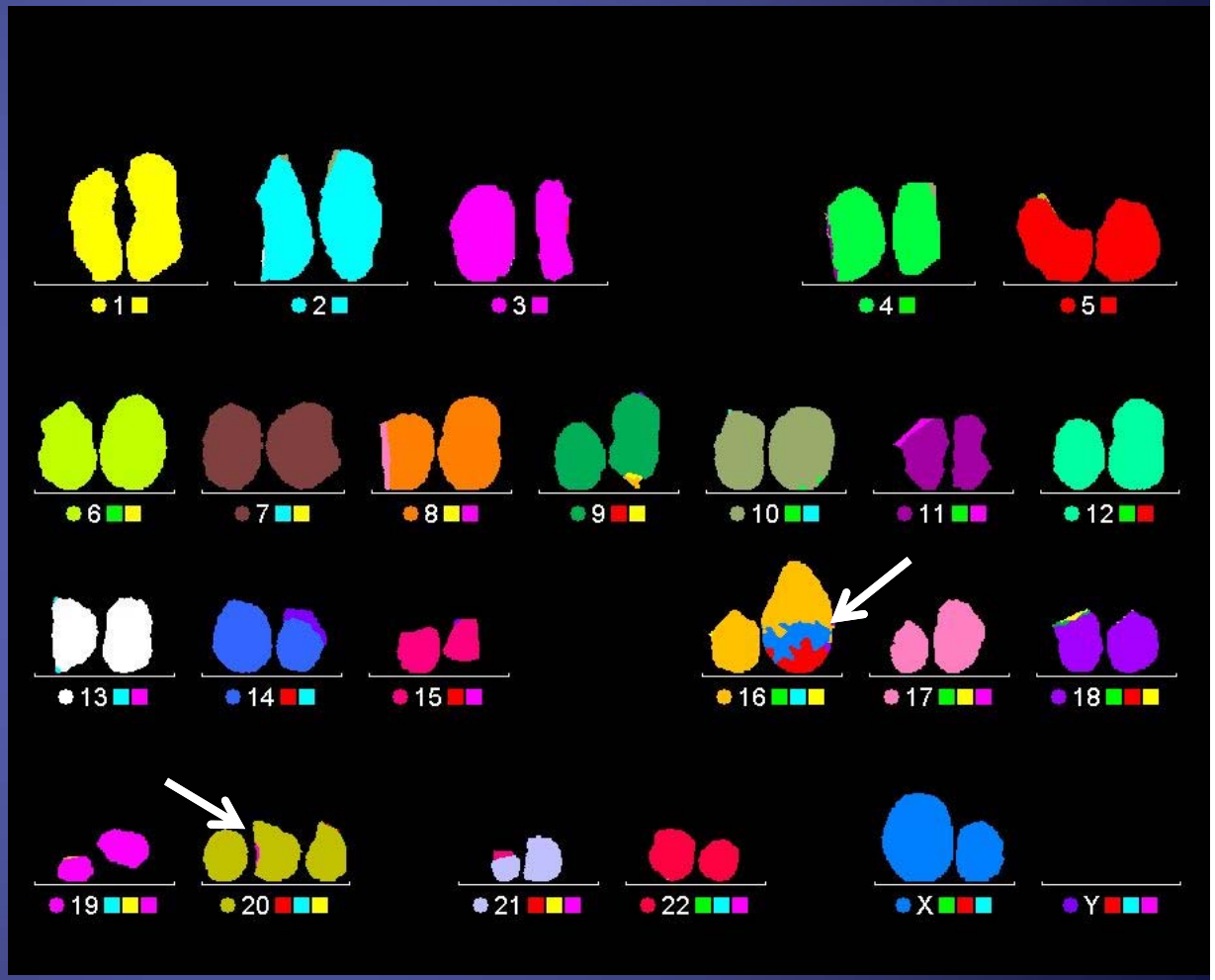
Result

Clone #1 unexposed

- Translocation occurs between chromosomes 16 and 5

- chromosome 20 has trisomy

- The chromosomes organization is similar to the parental clone



Result

Clone #2 unexposed

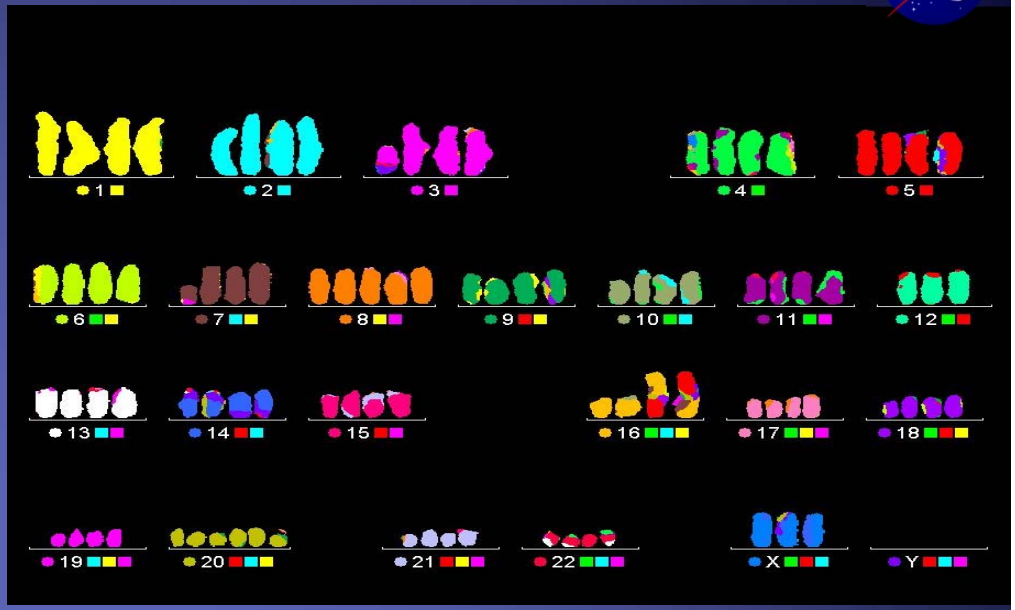
- Translocation that results in fusion between chromosome 3 and the X chromosome

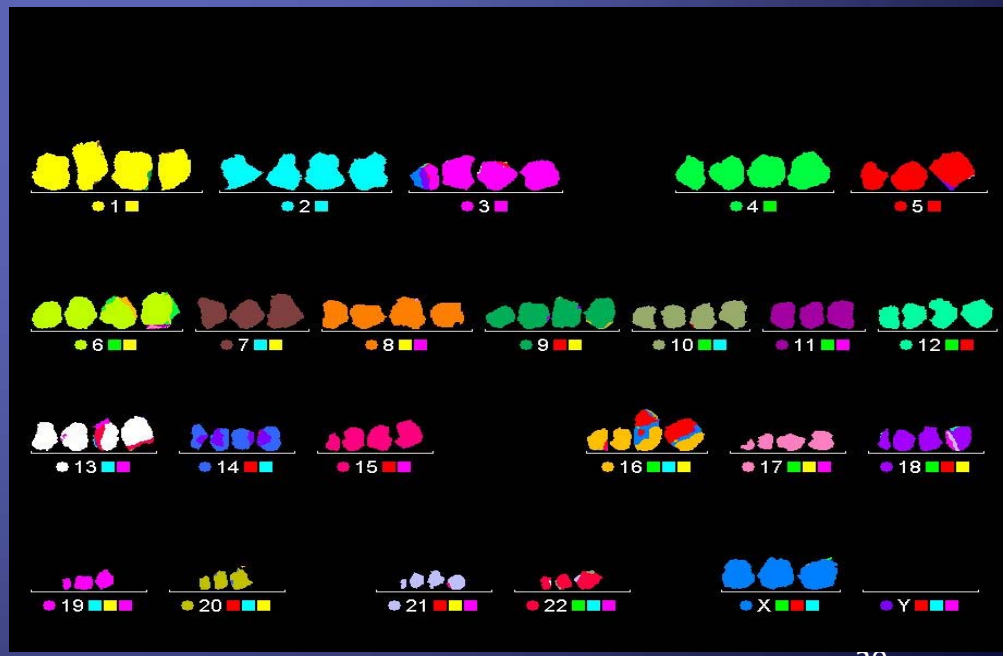
- The numbers of chromosomes observed double (i.e. 94 chromosomes) first cell

- Breakage of part of chromosome 7

- The numbers of chromosomes observed double (i.e. 84 chromosomes) the second cell

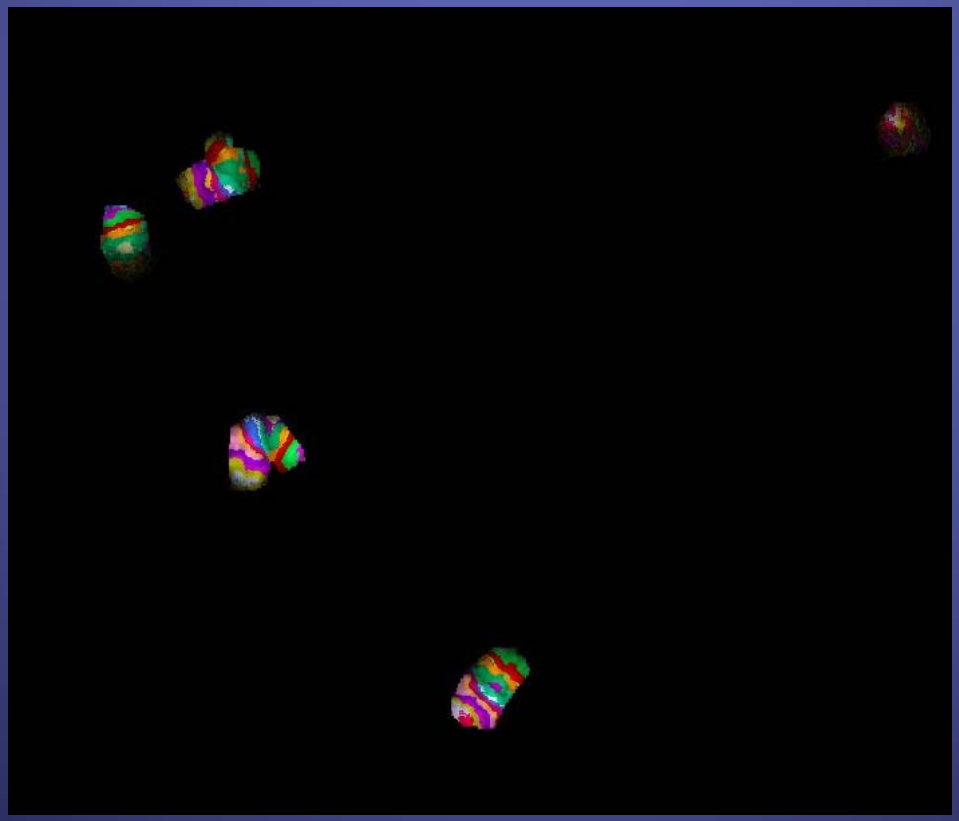
- It is likely that the DNA replicates without any cell division or it is likely that abnormal chromosomal segregation occurs during the anaphase of mitosis and few of the chromosomes are lost, such as chromosomes 5, 7, 11, 19, 20, 22 and the X-chromosome.





Clone #2
mBAND for chromosome 3

- **mBAND analysis showed breakpoint of chromosome involved in the translocation is near the centromere. Another breakpoint is near the telomere of the long arm.**



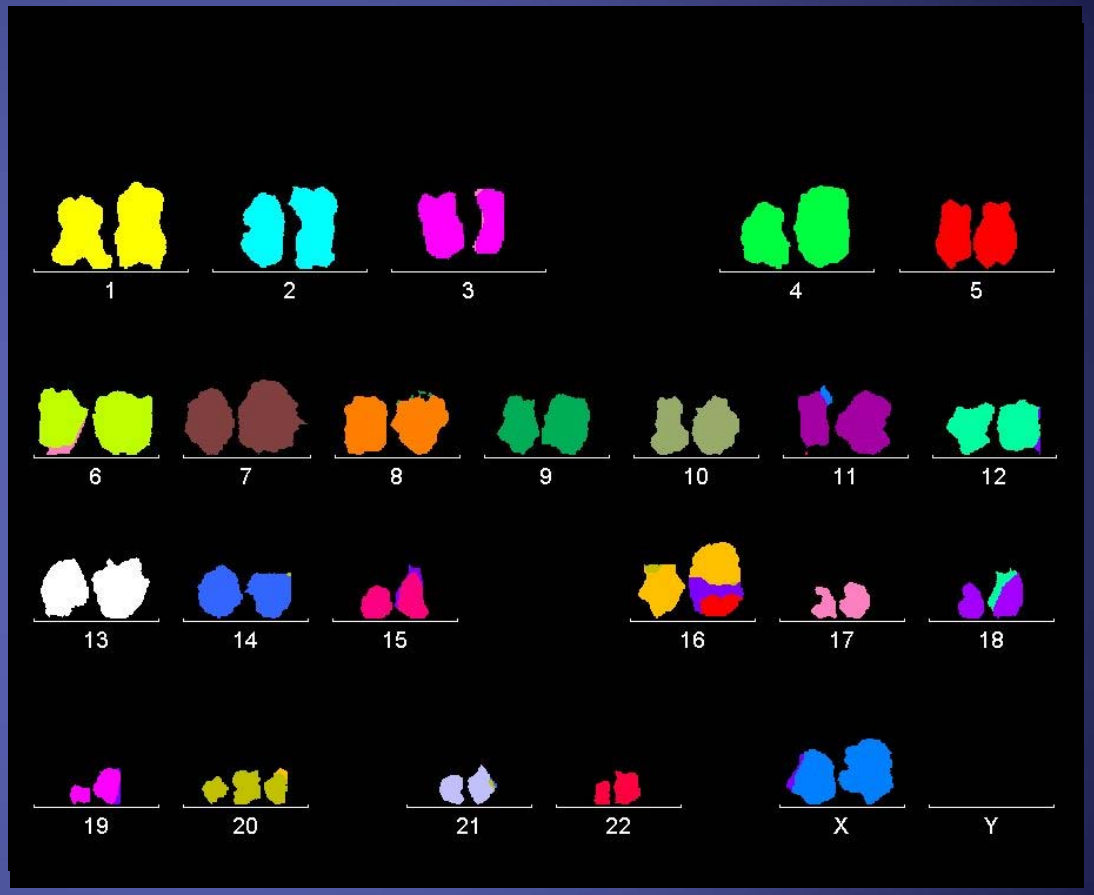
Result

Clone #3 irradiated

•Translocation that results in fusion between chromosome 5 and 16

• Chromosome 20 has trisomy

• One of the irradiated cells has the same chromosomal organization as the parental cells



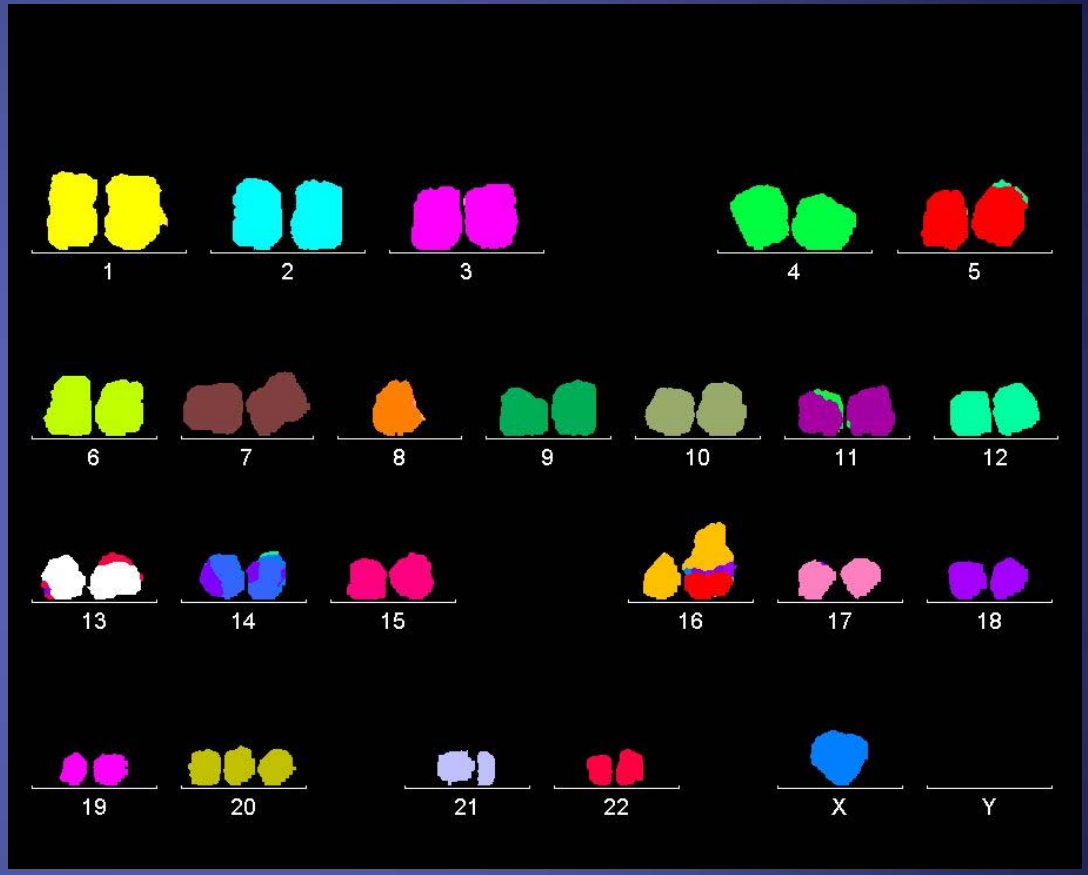
Result

Clone #4 irradiated

•Translocation between chromosome 16 and 5

• chromosome 20 has trisomy

• The number of total chromosomes in a cell nucleus appears to be less than 46.



Conclusions

- There is no different between the transformed parental cell and the single cloned transformed cell. Both harbor the chromosome 5 and 16 translocation and both harbor has the trisomy chromosome 20.
- Transformed cells may have the number of chromosomes greater or less than 46. Doubling of chromosome numbers is a signature of tumor.
- Chromosomal aberration was observed on HBEC-3kt non-irradiated-soft agar (Clone #2) sample, and indication of chromosome instability in the tumor development process.

Conclusions

- It is most likely that the untreated transformed single clone (clone #2)cell undergoes unequal segregation of chromosome in two daughter cell that result in 94 chromosome during mitosis, particularly in anaphase stage.
- Chromosome aberration observed

I. Breakage of part of chromosome 7

II. One additional number of chromosome 8 instead of the total chromosome can only be explain by early abnormal cell division.

III. Complete lost of chromosome and translocation and fusion of chromosome 3 and X-chromosome.

IV. Our result for translocation and fusion of chromosome 3 and X- Chromosome is conformed by mBAND pattern.

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